

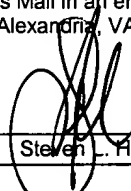
# FULBRIGHT & JAWORSKI L.L.P.

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October 18, 2004

CERTIFICATE OF MAILING 37 C.F.R. 1.8	
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<u>October 18, 2004</u> Date	 Steven L. Highlander

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-01450

Re: Serial Number 09/500,904 Entitled "DIAGNOSTICS AND THERAPY OF  
EPSTEIN-BARR VIRUS IN AUTOIMMUNE DISORDERS" By John B. Harley  
et al.; (Client Ref. No. OMRF:161)  
Our ref: OMRF:051US

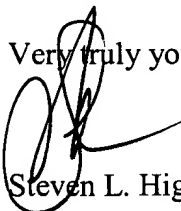
Commissioner:

Enclosed for filing in the above-referenced patent application is:

- (1) Inventors' Declaration Under 37 C.F.R. § 1.132; and
- (2) A return postcard to acknowledge receipt of these materials. Please date stamp and mail this postcard.

Should any fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason relating to the enclosed materials, the Commissioner is authorized to deduct said fees from Fulbright & Jaworski L.L.P. Account No.: 50-1212/OMRF:051US/SLH.

Very truly yours,

  
Steven L. Highlander

SLH/cpj  
Encl: As noted

25464524.1 / 10407564



PATENT  
CUSTOMER NO. 32425

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

John B. Harley *et al.*

Serial No.: 09/500,904

Filed: February 9, 2000

For: DIAGNOSTICS AND THERAPY OF  
EPSTEIN-BARR VIRUS IN  
AUTOIMMUNE DISORDERS

Group Art Unit: 1648

Examiner: S. FOLEY

Atty. Dkt. No.: OMRF:051US

CERTIFICATE OF MAILING  
37 C.F.R. § 1.8

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Box 1450, Alexandria, VA 22313-1450 on the date below:

October 18, 2004  
Date

Steven J. Highlander

**INVENTORS' DECLARATION UNDER 37 C.F.R. §1.132**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, Virginia 22313-1450

We, the undersigned, do declare that:

1. We are the John B. Harley, Kenneth M. Kaufman and Judith A. James named as inventors on the above-captioned application. John B. Harley is a citizen of the United States residing at 439 NW 20<sup>th</sup> Street, Oklahoma City, OK. Kenneth M. Kaufman is a

citizen of the United States residing at 708 NE 21<sup>st</sup> Street, Oklahoma City, OK. Judith A. James is a citizen of the United States residing at 701 NW 153<sup>rd</sup> Terrace, Edmond, OK.

2. We are also the John B. Harley, Kenneth M. Kaufman and Judith A. James named as authors on the attached manuscript entitled “An altered immune response to Epstein-Barr virus suggests a link to systemic lupus erythematosus,” by McClain *et al.* This manuscript has been submitted for publication and is presently under review.
3. In the manuscript, we describe studies designed to test whether the immune response to EBV acts as a risk factor for pediatric SLE and assesses the extent that the fine specificity of the anti-EBNA-1 response in SLE can be distinguished from normal control humoral responses. Pediatric lupus patient and healthy, matched control sera were tested to define the fine specificity of their anti-EBNA-1 humoral immune response utilizing a modified ELISA assay against the maximally overlapping octapeptides of EBNA-1.
4. Thirty-six SLE patients and their matched EBV positive controls were demonstrated to produce anti-EBV-VCA antibodies. All 36 SLE patients produced antibodies against a 70 kD band in the EBV infected lysates (from B95-8 or Jijoye), as well as by whole EBNA-1 ELISAs. Of the 36 sera from EBV-positive controls (matched on age, sex, and race), 11 (31%) did not produce detectable antibodies which recognized EBNA-1 from either the B95-8 or the Jijoye cell lines. Anti-EBNA-1 antibodies are therefore associated with SLE (OR=30.4,  $\chi^2=13.3$ ,  $p<0.005$ , CI 95% 1.7 to 544).

5. To explore differences in the humoral responses between cases and controls, expressed fragments containing the N-terminal (amino acids 1-89), middle (amino acids 90-330), and carboxyl terminal (amino acids 331-641) regions of EBNA-1 were tested. Twenty pediatric SLE patient sera and 20 sera from their matched, EBV-positive controls were randomly selected. Compared to the control sera, the SLE patients had relatively greater than average binding to the N-terminal fragment (containing the glycine-arginine rich segment) (mean OD=0.451 versus 0.268) and to the carboxy terminal fragment (OD=0.844 versus 0.296). Controls (OD=0.779) have higher average binding to the glycine-alanine rich middle segment (containing the multiple glycine-alanine repeats) when compared to the SLE patients (OD=0.396). All pairwise case-control comparisons are significant by student's T test ( $p < 0.001$ ).
6. The 20 lupus patient sera tested bound many different overlapping octapeptides of the EBNA-1 protein, while normal EBV-negative individuals do not. Binding of a representative SLE patient serum shows the many epitopes typically bound in the amino and carboxyl terminal regions of EBNA-1 in most of these pediatric SLE sera. In contrast, the anti-EBV-VCA positive normal control serum illustrates the stark differences usually observed between cases and controls, which is also demonstrated by the mean binding of SLE and their control sera. Anti-EBV-VCA negative control sera showed no significant reactivity (average background binding OD=0.118).
7. Among epitopes selectively bound by the SLE pediatric sera, amino acids 40-53 (GRGRGRGRGRGGR), known as the (GR<sub>X</sub>) region, and amino acids 398-404

(PPPGRRP) are commonly targeted, although this latter epitope just fails to achieve statistical significance when all SLE patients are averaged (binding is 1.98 standard deviations above the normal mean). However, all of these anti-PPPGRRP positive sera also bind Sm in the standard solid phase assay (data not shown). Some variation in the binding of individual SLE patient sera to these octapeptides does occur. For instance, about 67% of the SLE patient samples bind PPPGRRP. Tested pediatric SLE samples, however, do not significantly bind ( $>2$  S.D.), the regions comprising the (GA<sub>X</sub>) repeats that are the primary target of the normal humoral immune response.

8. In contrast the normal, EBV-positive pediatric controls reveal an entirely different binding pattern to the overlapping octapeptides of EBNA-1. Normal children and teenagers make antibodies primarily against two epitopes of the EBNA-1 protein (both of which are present in the middle recombinant EBNA-1 fragment). These epitopes consist of amino acids 101-113 (GGAGAGGGAGAGG) and amino acids 140-155 (GGAGAGGGAGAGGGAG), neither of which are recognized by the pediatric SLE patient sera. Furthermore, a group of five EBV-positive polymyositis patients show a nearly identical, limited response to the octapeptides of EBNA-1 as that seen in normal EBV-positive controls (data not shown). Unlike the SLE pediatric sera tested, non-SLE but EBV-positive individuals have a nearly uniform, almost monotonously replicated binding pattern, as is demonstrated by the close similarity between the representative example and mean binding. In fact, the mean binding pattern generally observed in the pediatric controls is different and virtually mutually exclusive from the pattern generated by the SLE pediatric patient sera. This result strongly suggests that the molecular and

cellular immunoregulatory events that lead to anti-EBNA-1 antibodies are significantly different in the pediatric SLE patients than they are in their normal pediatric matched controls.

9. CMV-IE was chosen as a control antigen since it is a DNA binding protein found in another life-long, common herpes virus infection and since CMV-IE is known to induce an antibody response in normal CMV-infected individuals. Both SLE patients and normal individuals recognize multiple, almost identical epitopes of CMV-IE. In contrast to the distinct qualitative differences between SLE and normal immune responses to EBNA-1, the SLE sera bind the same epitopes of the CMV-IE octapeptides less avidly than control sera.

10. We hereby declare that all statements made of our own knowledge are true and all statements made on information are believed to be true and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereon.

10/15/04  
Date

John B. Harley  
John B. Harley

10/15/04  
Date

Kenneth M. Kaufman  
Kenneth M. Kaufman

10/15/04  
Date

Judith A. James  
Judith A. James